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Original Article

A Six-year Analysis Report at the Cytogenetics Department of National Institute of Health Islamabad, Pakistan

Gulfaraz Khan[1], Jamil Ahmed Ansari[2], Muhammad Salman[3], Moazzam Abbas Ranjha[2], Faheem Tahir[4]
Department of Cytogenetics[1], Field Epidemiology and Disease Surveillance Division[2],Department of Virology[3], Public Health Laboratories Division[4], National Institute of Health, Islamabad.

Abstract

Background: The change in the number and structure of chromosomes result in chromosomal abnormalities, which can lead to serious medical problems in human beings. These problems may include delayed milestones in infants, presence of ambiguous genitalia, mental health problems and occurrence of repeated miscarriages in couples.
Objective: The study was carried out to see the frequencies of various chromosomal abnormalities in patients belonging to Rawalpindi and Islamabad cities, who were advised for chromosomal analysis.
Study design, settings and duration: A retrospective study was carried out at Cytogenetics Department of Public Health Laboratories Division in the National Institute of Health, Islamabad during January 2011 to December 2016.
Patients and Methods: A total of 363 patients, whose ages were between newborn to 38 years, were referred to Cytogenetics Department of Public Health Laboratories Division in the National Institute of Health, Islamabad during January 2011 to December 2016 (six years) with complaints of Down syndrome, Turner's syndrome, ambiguous genitalia and primary/ secondary amenorrhea. Karyotyping i.e. analysis of chromosomes of these patients was performed by lymphocyte culture technique. Data was collected on a pre-designed proforma by interviewing the case or his parents (if case was infant) and was entered in excel and analyzed with Epi-Info.
Results: Of the total 115 (31.6%) were males and 189 (52.2%) were females. Chromosomal abnormalities were found in 130 cases (35.8%) out of total 363 cases included in this study. Out of these abnormalities, Down's syndrome (46.6%) was the highest chromosomal abnormality followed by ambiguous genitalia (24.5%) and Turner syndrome (2.1%). However, among the total respondents (n=363) 49 females were presented with the complaint of primary Amenorrhoea and 19 with the complaint of Bad Obstetrics History (BOH) for chromosomal analysis. However, all 68 females had normal karyotypes.
Conclusion: From the results obtained in the above mentioned study, it transpires that chromosomal analysis plays an important role in investigating the chromosomal disorders in suspected patients and also helps physicians to confirm proper diagnosis of ailment and provide necessary counseling to the patients as well as their parents.
Key words: Down syndrome, turner syndrome, genetic abnormalities, incidence.

Introduction

Healthcare in Pakistan is provided by both public and private sectors. Public healthcare provision is insufficient and mostly utilized by people belonging to low socioeconomic backgrounds. This healthcare system however lacks specialized services for people with disabilities.[1] According to Statistics from Pakistan, the estimated population with genetic disorders is 29.2 million. Analysis showed that prevalence of such disorders is highest in Sindh while lowest in Punjab. Besides, the ratio of genetic disorder is higher in rural areas as compared to urban areas.[2]

Corresponding Author:
Gulfaraz Khan
Department of Cytogenetics
Public Health Laboratories Division
National Institute of Health, Islamabad.
Email: gulfarazkhan70@gmail.com

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Authors Contribution

GK, JAA & MS did conceptualization of study design & literature search. GK also did the data collection. GK, JAA & MAR performed the statistical analysis. Drafting, revision, writing of manuscript were done by GK, JAA, MS & FT.

A normal human being has 46 chromosomes. The change in the number and structure of chromosomes during cell division result in chromosomal abnormalities, which can lead to serious medical problems/ disabilities in human beings. These problems may include delayed milestones in infants, presence of ambiguous genitalia, mental health problems and occurrence of miscarriages in couples.[3]

Although chromosome abnormalities are of various types, however, they can be classified into two major groups' viz. numerical abnormalities and structural abnormalities. Numerical abnormalities are those abnormalities in which a certain number of chromosomes is missing in a person. For example, if a person is missing one of the chromosomes from a pair, this is called monosomy. An example of monosomy is Turner syndrome in which a patient is missing one chromosome and is characterized by short stature besides other difficulties. If a person is missing 2 or more than two chromosomes, it is called trisomy. An example of trisomy is Down syndrome in which the patient has three copies of chromosome 21 rather than two. Due to this abnormality, Down syndrome patients possess slanting eyes, depressed nasal bridge, low set ear and suffer from mental retardation.[4]

Structural abnormalities are those abnormalities in which a chromosome's structure is changed through different ways including deletions, duplications, translocations and inversions. When a certain portion of chromosome is deleted/ missed, the condition is called deletion whereas when a portion of chromosome is duplicated it is called duplication. In similarity, sometimes it happens so that a certain portion of a chromosome is transferred and attached to another chromosome resulting in translocations while inversions may also occur through formation of a circle or ring by broking off and turning upside of a chromosomes. Majority of these abnormalities took place accidentally in the egg or sperm. If it happens so, then the abnormality occurs in every cell of the body. A person may get such chromosomal abnormalities from his parent as well that is why when a child is having an abnormality, chromosomal studies are also advised to both of his parents.[5]

Factors like consanguinity (inter-cousin marriages) and parental age can contribute in the chances of chromosome abnormalities in the off-springs. Pregnancies at older ages can give birth to babies with chromosomal abnormalities. As such, medical genetic disorders are the consequences of chromosomal abnormalities. Resultantly, the patients with delayed milestones, presence of ambiguous genitalia, mental problems and spontaneous miscarriages in couples are referred for karyotyping by the clinicians/ physicians.[6]

For chromosome analysis, karyotyping is advised, which is an ordered pairing of homologous chromosomes.[7] The changes in the number or structure of the chromosomes can be identified with the help of this analysis (Karyotyping). These changes may be responsible for diseases characterized by Down syndrome, infertility/sterility (e.g. Turner and Klinefelter syndromes), growth and development. Even the exposure to early abortion may be as a result of a chromosomal error in one of the parents. The karyotyping is performed in patients with suspected chromosomal syndrome, mental retardation and / or birth defects, retardation, in infants; born or dead, couples with repeated miscarriages, male infertility and women with absence or interruption of the menstrual cycle.[8]

This retrospective study was carried out to see the frequencies of various chromosomal abnormalities in patients belonging to Rawalpindi-Islamabad region because very few studies have been conducted on this topic as the technique used i.e. karyotyping is not a common and is being carried out only in few laboratories like Armed Forces Institute of Pathology (AFIP), Rawalpindi, Agha Khan University Hospital, Karachi, KRL Hospital Islamabad and Excel Labs (Pvt.), Islamabad etc.

Patients and Methods

A total of 363 patients ranging from newborn to 38 years of age referred to Cytogenetics Department of Public Health Laboratories Division in the National Institute of Health, Islamabad during January 2011 to December 2016 with complaints of Down syndrome, Turner's syndrome, ambiguous genitalia and primary/ secondary amenorrhea were included in this study to see and identify the burden of these diseases. The factors of parental age and consanguinity (inter-cousin marriages) amongst parents were also considered in these diseases.

Depending upon the nature of their ailment, the patients were divided into four groups: a) Down's syndrome (presented with specific facial appearance like slanting eyes, depressed nasal bridge, clindodactyl, single line in the palm etc; b) Turner syndrome (presented with features like short stature, webbed neck, & widely spaced nipples etc; c) Ambiguous genitalia (presented with undifferentiated genital organs into male or female etc); and d) Primary/ secondary Amenorrhoea and miscellaneous group having delayed milestones and intellectual disability etc.[3]

All cases referred to NIH from public and private hospitals and clinics of Rawalpindi/ Islamabad including Federal Government Polyclinic Hospital, Pakistan Institute of Medical Sciences, Capital Development Authority Hospital, Benazir Bhutto Hospital, Holy family hospital, Federal General Hospital and private clinical labs of Islamabad/ Rawalpindi irrespective of age and gender were included in the analysis. No duplicate sample was included. Permission to carry out this study was taken from the Ethical Review Committee of PHLD, NIH. The information was collected on a pre-designed proforma by interviewing the case or his parents (if case was infant). This information includes nature of ailment, physical features, clinical picture, parental age, consanguinity, pregnancy history and family history of the patients.

The information of all cases was entered in excel and analyzed with Epi-Info. The statistical analysis has also been done to classify the data.

For cytogenetic analysis, about 3cc whole blood of patient was collected in sodium heparin blood collection tube. Chromosomal analysis was performed on cultured lymphocytes for which culture medium was prepared by mixing specific proportions of RPMI 1640 (in liquid form), Fetal Bovine Serum (FBS), Phytohaemagglutinin (PHA), L. Glutamine and Penicillin Streptomycin (PSS). Five (05) ml of complete medium was taken into a culture tube. Then 0.5 ml of blood was added in the tube (culture). The culture was shaken and incubated at 37°C for 72 hrs. The contents of tube were mixed twice a day to guard against clumping. After incubation, mitosis was arrested by adding 0.2ml colchicines to the culture. The culture was again incubated for 1.5-2 hrs at 37°C. The culture was then spinned at 1000 revolution per minute (rpm) for 10 minutes. The supernatant was discarded and 05 ml of hypotonic solution of KCl was added, mixed gently and left at 37°C for 30 minutes. After that 2-3 drops of freshly prepared fixative (3 parts of methanol +1 part of acetic acid) were added and centrifuged at 1000 rpm for 10 minutes. The supernatant was again discarded and four (04) ml of freshly prepared fixative was added. The pellet of cells was re-suspended gently and carefully by means of a pasteur pipette and centrifuged at 1000 rpm for 10 minutes. The supernatant was again discarded remaining behind the pellet or sediment at the bottom of the tube. Finally, slides were prepared from the sediment, stained with Giemsa stain and visualized under microscope for chromosomal counting/ karyotyping.[9,10] Data was entered in excel and analyzed with Epi-Info to calculate frequencies of different genetic disorders. Genetic counseling was provided to those patients in which chromosomal abnormalities were found.

Results

Out of 363 cases evaluated in this study, 115 (31.6%) were males, 189 (52.2%) were females and 59 (16.2%) were presented with ambiguous genitalia. Chromosomal abnormalities were found in 130 cases (35.8%).

Out of these 363 submitted cases, 142 were children under 6 months and 38 were above 6 months to 1 year. The age and developmental stage wise distribution of cases is summarized in Table-1.

Table 1: Age and developmental stage wise distribution of cases. (N= 363)

Ages	*Developmental Stage*	*Cases*	*%*
1 day - Below 6 months	Infants	142	39.12
6 months - 1 year	Infants	38	10.47
Above 1 year - 5 years	Child	60	16.53
Above 5 years - 10 years	Child	21	5.79
Above 10 years - 15 years	Adolescents	27	7.44
Above 15 years - 20 years	Adolescents	36	9.92
Above 20 years - 30 years	Adult	32	8.82
Above 30 years	Adult	7	1.93
Total		363	100

Amongst the total cases (n=363), 238 were presented with suspicion of Down's Syndrome out of which 111 (46.6%) were confirmed for Down's Syndrome. Among these 111 cases, 63 (56.8%) males as compared to 48 (43.2%) female were confirmed as Down's Syndrome (Table-2).

Among the total cases (n=363), 59 were presented with complaint of ambiguous genitalia and among them 14 (23.7%) were lab confirmed for ambiguous sex. Eleven females were presented with ambiguous sex and diagnosed as male while 3 females were presented with ambiguous sex and diagnosed as male (Table-2).

Table 2: Gender-wise frequency, median age and inter quartile range for the disease cases.

Diseases	*No of cases*	*%*	*Median Age*	*Inter Quartile Range (IQR)*
Down syndrome	111 (63 M & 48 F)	46.60	5 Mon	1 Mon to 1 Year
Ambiguous sex	14 (11 M & 3 F)	23.70	3.5 Mon	25 Days to 2.3 Years
Turner syndrome	5 (All Females)	2.10	2 Mon	20 Days to 10 Years

Among the total cases (n=363), 238 were presented with the complaint of Turner Syndrome and 5 (2.1%) remained lab confirmed for Turner Syndrome. All the cases having turner syndrome were female. Median age of the cases of turner syndrome was 2 months (Table-2).

Among the total cases (n=363) 49 females were presented with the complaint of Primary Amenorrhoea and 19 with the complaint of Bad Obstetrics History (BOH) for chromosomal analysis. However, all 68 females had normal karyotypes.

Out of the total 363 cases were evaluated, 221 (60.88%) cases have parental consanguinity. Out of these 221 cases, 50 (22.62%) were positive for Down Syndrome and 2 (0.90%) were positive for Turner Syndrome. The year-wise distribution of cases with parental consanguinity, Down syndrome and Turner syndrome is represented in Figure-1.

The age groups of parents of cases are summarized in a frequency distribution in Table-3. Out of 363 cases mothers of 275 cases reported their ages. 51 (18.55%) mothers were in the age group 15 years to 24 years.

Out of 363 cases, fathers of 273 cases reported their ages. 11 (4.03%) fathers were in the age group 15 years to 24 years.

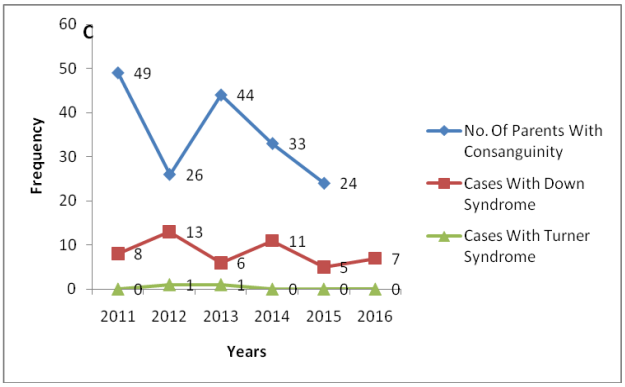
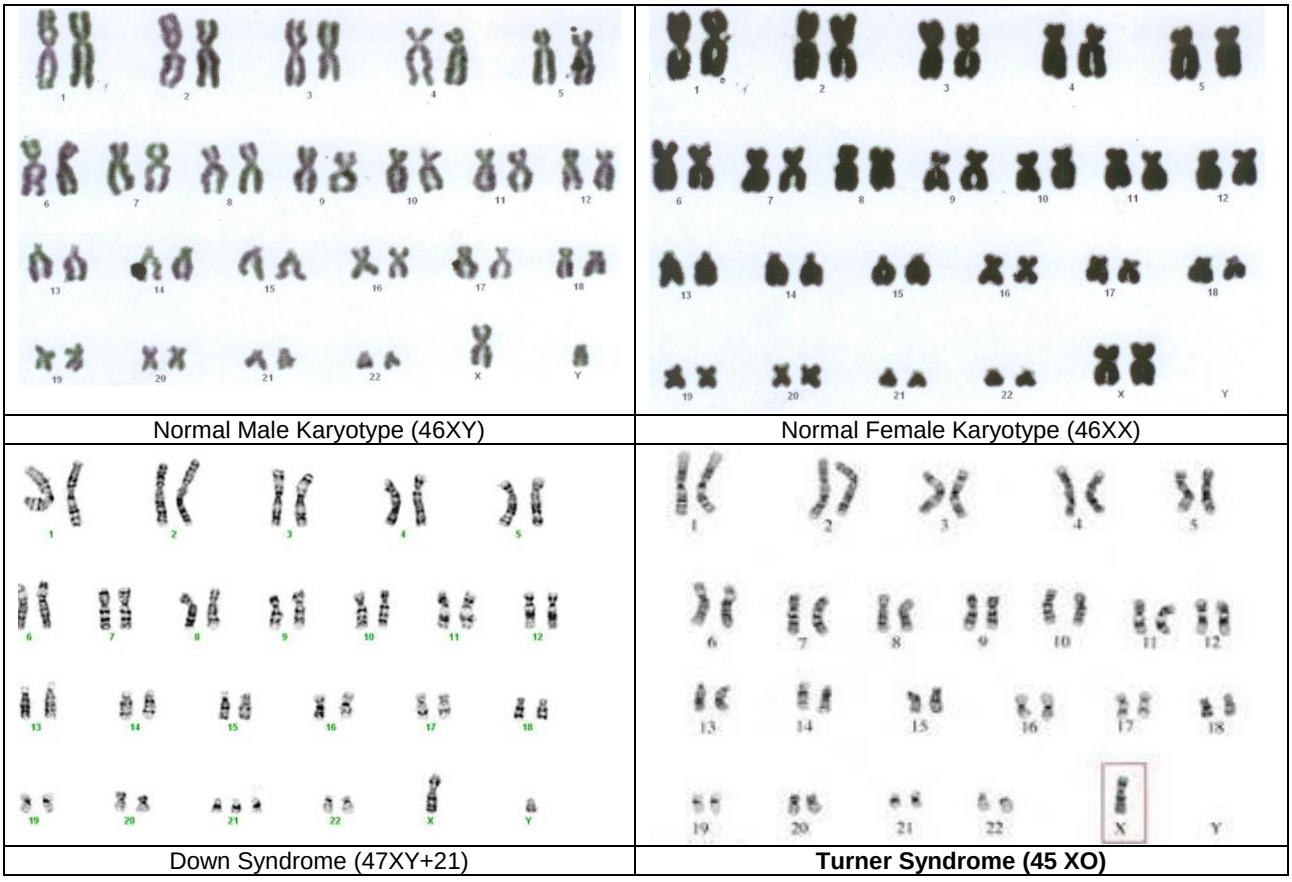


Figure 1:Cases with down syndrome, tumer syndrome having consanguinity.



Normal Male Karyotype (46XY)	Normal Female Karyotype (46XX)
Down Syndrome (47XY+21)	**Turner Syndrome (45 XO)**

Figure 2: Karyotypes of a normal male, normal female, a down syndrome and turner syndrome patient.

Table 3: Frequency Distribution for the age of parents of cases.

Age Groups (Years)	Mothers		Fathers	
	F	%	F	%
15-24	51	18.55	11	4.03
25-34	149	54.18	134	49.08
35-44	57	20.73	86	31.50
45-54	12	4.36	32	11.72
55-64	4	1.45	5	1.83
65-74	2	0.73	5	1.83
Total	275	100	273	100

Out of the total 363 cases, 50 cases with Down syndrome had parents with consanguinity. The frequencies of parental consanguinity and Down syndrome are given by a cross table in Table-4. According to the cross table the number of cases of Down syndrome without parental consanguinity were more than the cases of Down syndrome with parental consanguinity.

Table 4: Cross table for parental consanguinity and cases with down syndrome.

Disease\ Consanguinity	Parents with Consanguinity (%)	Parents without Consanguinity (%)	Total
Cases having down syndrome	50	61	111
Cases without down syndrome	171	81	252
Total	221	142	363

Four karyotypes viz, normal male (46XY), normal female (46XX), a Down syndrome (47 XY+21) and a Turner Syndrome (45XO) have been presented in Figure-2.

Discussion

Most of the genetic abnormalities are directly linked with chromosomal aberrations. Cytogenetics deals with the structure and properties of chromosomes and cell division. It employs various methods including karyotyping in which chromosomes are arranged in a standard manner. Through this technique, undetected chromosomal anomalies can be seen like small portions of chromosomes and translocations of their tiny parts to one another. As each pair of chromosomes can be distinguished individually by virtue of this technique, it helps to better understand chromosomal basis of certain important genetic disorders.[18]

Pakistan is a developing country and has limited resources to cater the needs of its vast health care system. The majority of the population of this country lives in rural areas where very meager or no facility is available for antenatal monitoring.[15] Chromosomal abnormalities including Down syndrome account for a vital public health problem as the affected children need extra care and special attention to cope with their problems to lead a healthy life.[16] As described above in the results, chromosomal abnormalities were found to be 35.8% in this study. Similar results were revealed in a study published in India where chromosomal abnormalities were reported to be 42.4%.[3]

The current study reveals that the most common type of chromosomal disorder (46.6%) was found to be Down syndrome (Trisomy 21) amongst children of median age of 5 months. Down syndrome abnormality is found most commonly in the infants, which can lead to serious problems including respiratory, hematological & cardiac diseases.[16]

The incidence of ambiguous sex or inter-sexuality i.e. undifferentiated genital organs into male or female was considerable (24.5%) with a median age of 3.5 months. Our study, provided the similar results as compared with other studies conducted on cases suspected of having genetic disorders which showed similar frequencies of such chromosomal abnormalities.[11-13]

The current study provided that the frequency of Turner syndrome was very low i.e. 2.1%. In Turner syndrome, one chromosome in a patient is missing. Similar results were reported regarding prevalence of Turner syndrome in a study published in India.[14]

Consanguinity is one of the major causes of genetic disorders in Pakistan where about 61.3% of marriages are consanguineous. However, most of the Pakistani population is unaware of this fact. It is anticipated that 700 children are born every year with genetic disorders owing to cousin marriages. In our study 50 (22.62%) cases positive for Down syndrome and 2 (0.90%) positive for Turner syndrome out of the 221 cases belong to parental consanguinity. Moreover, children born from first cousin marriages are a greater risk of miscarriage or infertility.[2]

The current study also reveals frequency of Down syndrome more frequently in our society. As there is no way to prevent Down syndrome, therefore in order to reduce the incidence of this disease, health care providers need to promote necessary genetic counseling services to the parents. However, with the recent advancement in the medical field it has now become possible for the

Down syndrome people to lead a healthy and normal life by easing the burden of their family members.[17]

Genetic analysis is an essential tool, which can make the couples aware about the reappearance of such chromosomal abnormalities in their future pregnancies. Genetic counseling also helps families to cope up with emotional, psychological and medical consequences of genetic disorders.[3] Further progress in the primary prevention of Down syndrome is hampered by limited knowledge of the cause of this disorder. Therefore, there is an urgent need to refocus research in that direction.

Literature search showed that there is a dearth of research studies in Pakistan on Down syndrome. Only few studies were found on genetic disorders including Down syndrome in Pakistan. Basic statistics pertaining to prevalence, incidence ratios and demographics of Down syndrome population has not yet been reported in Pakistan.[19] Moreover, lack of public health awareness genetic counselling and health care before and during pregnancy also exists in Pakistan. Hence appropriate platforms for genetic counseling genetic screening and pre-natal diagnosis are required to be established.[2]

In short, karyotyping plays a vital role in genetic counselling. This can help individuals/ parents to make personal decisions about their health, future pregnancies or their child's health care if they themselves have a genetically determined disorder or are at risk. The benefit of karyotype analysis in high risk population therefore provides the prevention and early management options to minimize the risk.[20]

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